FORM PTO-1390 (Modified) (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

# TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER: **110.00680101**

U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR): **09/529691**

| INTERNATIONAL APPLICATION NO. | INTERNATIONAL FILING DATE | PRIORITY DATE CLAIMED |
|---|---|---|
| **PCT/US98/22405** | **22 October 1998** | **22 October 1997** |

TITLE OF INVENTION

**INHIBITION OF TUMOR CELL ADHESION TO TYPE IV COLLAGEN**

APPLICANT(S) FOR DO/EO/US

**Gregg B. FIELDS and James B. McCARTHY**

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☐ A copy of the International Search Report (PCT/ISA/210).
8. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
9. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
10. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371 (c)(4)).
11. ☐ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).

**Items 13 to 20 below concern document(s) or information included:**

13. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☒ Certificate of Mailing by Express Mail
20. ☒ Other items or information:

**Signed verified statement of small entity status.**

| U.S. APPLICATION NO. (IF KNOWN, SEE 37 CFR) | INTERNATIONAL APPLICATION NO. | ATTORNEY'S DOCKET NUMBER |
|---|---|---|
| 09/529691 | PCT/US98/22405 | 110.00680101 |

21. The following fees are submitted:

| BASIC NATIONAL FEE ( 37 CFR 1.492 (a) (1) - (5)) : | | CALCULATIONS PTO USE ONLY |
|---|---|---|
| ☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2) paid to USPTO and International Search Report not prepared by the EPO or JPO | $970.00 | |
| ☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but Internation Search Report prepared by the EPO or JPO | $840.00 | |
| ☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO | $690.00 | |
| ☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) | $670.00 | |
| ☐ International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) | $96.00 | |
| **ENTER APPROPRIATE BASIC FEE AMOUNT =** | | $840.00 |
| Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)). | | $130.00 |

| CLAIMS | NUMBER FILED | NUMBER EXTRA | RATE | |
|---|---|---|---|---|
| Total claims | 24 - 20 = | 4 | x $18.00 | $72.00 |
| Independent claims | 2 - 3 = | 0 | x $78.00 | $0.00 |
| Multiple Dependent Claims **(check if applicable)**. | | | ☒ | $260.00 |
| **TOTAL OF ABOVE CALCULATIONS** | | | = | $1,302.00 |
| Reduction of 1/2 for filing by small entity, if applicable. Verified Small Entity Statement must also be filed (Note 37 CFR 1.9, 1.27, 1.28) **(check if applicable)**. | | | ☒ | $651.00 |
| **SUBTOTAL** | | | = | $651.00 |
| Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). | | | + | $0.00 |
| **TOTAL NATIONAL FEE** | | | = | $651.00 |
| Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** | | | ☐ | $0.00 |
| **TOTAL FEES ENCLOSED** | | | = | $651.00 |
| | | | **Amount to be: refunded** | $ |
| | | | **charged** | $ |

☒ A check in the amount of **$651.00** to cover the above fees is enclosed.

☐ Please charge my Deposit Account No. in the amount of to cover the above fees.
A duplicate copy of this sheet is enclosed.

☒ The Commissioner is hereby authorized to charge any fees which may be required, or credit any overpayment to Deposit Account No. **13-4895** A duplicate copy of this sheet is enclosed.

**NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.**

SEND ALL CORRESPONDENCE TO:

**Ann M. Mueting**
**MUETING, RAASCH & GEBHARDT, P.A.**
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Ann M. Mueting
SIGNATURE

**Ann M. Mueting**
NAME

**33,977**
REGISTRATION NUMBER

April 18, 2000
DATE

PATENT
Attorney Docket No. 110.00680101

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

| | | |
|---|---|---|
| Applicant(s): | Gregg B .Fields, et al. | ) |
| | | ) |
| Serial No.: | Unknown | ) |
| | | ) |
| Filed: | Concurrently Herewith | ) |
| | | ) |
| For: | INHIBITION OF TUMOR CELL ADHESION TO TYPE IV COLLAGEN | |

**PRELIMINARY AMENDMENT**

BOX PCT
Assistant Commissioner for Patents
Washington, D.C. 20231

Prior to examination and consideration of the above-identified U.S. national phase patent application, Applicant respectfully requests entry of the following amendments to the application:

**IN THE SPECIFICATION**

On page 1, line 8, before the heading "Background of the Invention", please insert the following new paragraphs:

-- **Cross-Reference to Related Applications**

This application is a national stage filing of International Patent Application No. PCT/US98/22405, filed on October 22, 1998; which in turn is an international filing of U.S. Provisional Patent Application No. 60/062,617, filed on October 22, 1997, and of U.S. Provisional Patent Application No. 60/062,716, filed on October 22, 1997.

**Statement Regarding Government Support**

The invention was developed under the support of Grant No. DK44494 (National Institute of Diabetes and Digestive and Kidney Diseases), Grant No. AR01929 (National Institute of Arthritis and Musclo-Skeletal and Skin Diseases), and Grant No. CA63671 (National Institutes of Health). The government may have certain rights to the invention. --

## IN THE CLAIMS

Please amend claim 19 as follows:

19. (AMENDED) The method of [any of claims 16-18] claim 16 which is carried out *in vivo*.

Please add new claims 20-21 as follows:

20. (NEW) The method of claim 17 which is carried out *in vivo*.

21. (NEW) The method of claim 18 which is carried out *in vivo*.

## REMARKS

The amendments to the description are made to add a cross-reference to related applications and to insert a statement regarding U.S. government support. The amendments to the claims are made to modify a multiple dependency to accord with U.S. rules of practice.

If the Examiner wishes to discuss any issues concerning this communication by telephone, please contact the below-signed attorney.

Respectfully submitted,

GREGG B. FIELDS, ET AL.

By Applicant's Representatives,

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April 18, 2000
Date

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AMM/mi

"Express Mail" mailing label number EL196578087US Date of Deposit April 18, 2000
I hereby certify that this paper and/or fee is/are being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 C.F.R. 1.10 on the date indicated above and is addressed to the Assistant Commissioner for Patents, Attn: Box PCT, Washington, D. C. 20231

Name: MARC IRELAND

Docket No. 110.0068 0101

# NONPROFIT ORGANIZATION

VERIFIED STATEMENT (DECLARATION) CLAIMING SMALL ENTITY STATUS
(37 C.F.R. §§1.9(f) AND 1.27(d)) - NONPROFIT ORGANIZATION

I hereby declare that I am an official empowered to act on behalf of the nonprofit organization identified below:

NAME OF ORGANIZATION: REGENTS OF THE UNIVERSITY OF MINNESOTA
ADDRESS OF ORGANIZATION: 600 Gateway, 200 Oak Street SE
Minneapolis, MN 55455

TYPE OF ORGANIZATION:

a) (X) UNIVERSITY OR OTHER INSTITUTION OF HIGHER EDUCATION
b) ( ) TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 U.S.C. §§501(a) AND 501(c)(3))
c) ( ) NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA -- (NAME OF STATE _____)
(CITATION OF STATUTE _____)
d) ( ) WOULD QUALIFY AS TAX EXEMPT UNDER INTERNAL REVENUE SERVICE CODE (26 U.S.C. §§501(a) AND 501(c)(3)) IF LOCATED IN THE UNITED STATES OF AMERICA
e) ( ) WOULD QUALIFY AS NONPROFIT SCIENTIFIC OR EDUCATIONAL UNDER STATUTE OF STATE OF THE UNITED STATES OF AMERICA IF LOCATED IN THE UNITED STATES OF AMERICA -- (NAME OF STATE _____)
(CITATION OF STATUTE _____)

I hereby declare that the nonprofit organization identified above qualifies as a nonprofit organization as defined in 13 C.F.R. §1.9(e) for purposes of paying reduced fees under Sections 41(a) and (b) of Title 35, United States Code, in regard to the invention, entitled INHIBITION OF TUMOR CELL ADHESION TO TYPE IV COLLAGEN by inventor(s) Gregg B. Fields and James B. McCarthy described in

a) (X) the specification filed herewith.
b) ( ) application serial no. _____, filed _____.
c) ( ) patent no. _____, issued _____.

I hereby declare that rights under contract or law have been conveyed to and remain with the nonprofit organization with regard to the above-identified invention.

If the rights held by the nonprofit organization are not exclusive, each individual, concern, or organization having rights to the invention is listed below* and no rights to the invention are held by any person, other than the inventor(s), who could not qualify as an independent inventor(s) under 37 C.F.R. §1.9(c) or by any concern that would not qualify as a small business concern under 37 C.F.R. §1.9(d) or a nonprofit organization under 37 C.F.R. §1.9(e). *NOTE: Separate verified statements are required from each named person, concern, or organization having rights to the invention averring to their status as small entities. (37 C.F.R. §1.27)

NAME _____
ADDRESS _____
( ) INDIVIDUAL ( ) SMALL BUSINESS CONCERN ( ) NONPROFIT ORGANIZATION

NAME _____
ADDRESS _____
( ) INDIVIDUAL ( ) SMALL BUSINESS CONCERN ( ) NONPROFIT ORGANIZATION

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 C.F.R. §1.28(b))

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application, any patent issuing thereon, or any patent to which this verified statement is directed.

NAME Michael F. Moore
TITLE Director - Health Technologies
ADDRESS 450 Gateway, 200 Oak Street SE, Minneapolis, MN 55455

SIGNATURE _____ DATE April 17, 2000

# INHIBITION OF TUMOR CELL ADHESION TO TYPE IV COLLAGEN

## Background of the Invention

Type IV collagen is a distinctive glycoprotein which occurs almost exclusively in basement membranes, structures which are found in the basal surface of many cell types, including vascular endothelial cells, epithelial cells, etc. Type IV collagen has a molecular weight (MW) of about 500,000 and consists commonly of two $\alpha$1 (MW 185,000) chains and one $\alpha$2 (MW 170,000) chain. Type IV collagen has two major proteolytic domains: a large, globular, non-collagenous, NCl domain and another major triple-helical collagenous domain. The latter domain is interrupted by non-collagenous sequences of variable length. It is a complex and multidomain protein with different biological activities residing in different domains.

Type IV collagen self-assembles to polymeric structures which constitute the supportive frame of basement membranes. Various macromolecular components bind to type IV collagen, such as laminin, entactin/nidogen, and heparin sulfate proteoglycan. An additional function of type IV collagen is to mediate cell binding. A variety of cell types specifically adhere and spread onto type IV collagen-coated substrata. Various cell surface proteins, a 47 kD protein, a 70 kD protein, and members of the superfamily of integrins have been reported to mediate cell binding to type IV collagen.

Several synthetic peptides derived from the triple-helical region of type IV collagen are known to support cell adhesion and motility (G.B. Fields, *Connect. Tissue Res., 31*, 235-243 (1995)). A peptide incorporating $\alpha$1(IV) residues 1263-1277 and designated IV-H1 has been demonstrated to support melanoma cell adhesion (U.S. Patent No. 5,082,926 (Chelberg et al.); M.K. Chelberg et al., *J. Cell. Biol., 111*, 261-270 (1990); K. Mayo et al., *Biochemistry, 30*, 8251-8267 (1991); and C.G. Fields et al., *J. Biol. Chem., 268*, 14153-14160 (1993)). IV-H1 also supports melanoma cell motility and selectively inhibits cell adhesion to type IV collagen (M.K. Chelberg et al., *J. Cell. Biol., 111*, 261-270

(1990)). Melanoma cell motility is mediated by a chondroitin sulfate proteoglycan (D.J. Mickelson et al., *J. Cell. Biol.*, *115*, 287a (1991)) and dependent upon IV-H1 conformation (M.K. Chelberg et al., *J. Cell. Biol.*, *111*, 261-270 (1990); K. Mayo et al., *Biochemistry*, *30*, 8251-8267 (1991)). However, these studies involved the all-L form of the polypeptide.

There is no general corrolary that all-D forms of peptides will function in the same manner as all-L forms. D-amino acid substituted analogs of a Gly-Arg-Gly-Asp-Ser-Pro peptide have been studied for inhibition of rat kidney cell adhesion to either fibronectin (via the $\alpha_5\beta_1$ integrin) or vitronectin (via the $\alpha_v\beta_3$ integrin) (M.D. Pierschbacher et al., *J. Biol. Chem.*, *267*, 14118-14121 (1992)). Substitution of Arg with D-Arg had no effect on the inhibitory activities of the peptide, while substitution of Asp with D-Asp resulted in an inactive peptide. Thus, inhibition of integrin binding to either fibronectin or vitronectin by Arg-Gly-Asp sequences is sensitive to the peptide inhibitor stereochemistry. Additional studies which correlated the NMR-derived structures of cyclic Arg-Gly-Asp analogs with inhibition of $\alpha_v\beta_3$ integrin binding to vitronectin indicated that the $\alpha_v\beta_3$ integrin interacts with both the Arg-Gly-Asp peptide side-chains and backbone (J. Wermuth et al., *J. Am. Chem. Soc.*, *119*, 1328-1335 (1997)).

In contrast, the laminin derived synthetic peptide LAM-L (A chain residues 2097-2108) and its all D-enantiomer had near identical concentration-dependent activities for promotion of rat pheochromocytoma cell (PC12) attachment, inhibition of PC12 adhesion to laminin, and promotion of murine melanoma cell growth in mice (M. Nomizu et al., *J. Biol. Chem.*, *267*, 14118-14121 (1992)). The cell surface receptor for LAM-L or LAM-D was not identified. A synthetic combinatorial library has been used to select an all-D peptide (acetyl-Arg-Phe-Trp-Ile-Asn-Lys-$NH_2$) as a potent ligand for the $\mu$ opioid receptor (C.T. Dooley, *Science*, *266*, 2019-2022 (1994)). The peptide was shown to be a full agonist, binding to the $\mu$ receptor and inducing a conformational change which allowed for signal transduction. In this case, the all-L peptide was not active.

Schnolzer and Kent (M. Schnolzer et al., *Science*, *256*, 221-225 (1992)) synthesized all-L and all-D HIV-1 proteases, then examined the chiral specificity of the two enzymes using the substrate 2-aminobenzoyl-Thr-Ile-Nle-Nph-Gln-Arg-$NH_2$ (where Nph is nitrophenylalanine). The synthetic all-L enzyme cleaved only the all-L, not the all-D, version of 2-aminobenzoyl-Thr-Ile-Nle-Nph-Gln-Arg-$NH_2$ while the synthetic all-D enzyme cleaved only the all-D substrate. The chiral specificity of enzymes was established by these results.

The results of other enzyme studies are consistent with those from the HIV-1 study, in that native (all-L) enzymes cleave only all-L substrates, not all-D substrates. For example, trypsin cleaves all-L cecropin A but does not cleave all-D cecropin A (D. Wade et al., *Proc. Natl. Acad. Sci. USA*, *87*, 4761-4765 (1990)). Further, trypsin cleaved L-Hep-III rapidly but did not hydrolyze D-Hep-III (C. Li et al., *Biochemistry*, *36*, 15404-15410 (1997)).

## Summary of the Invention

The present invention provides polypeptides which represent an all-D form of a fragment of the $\alpha$1 chain of human type IV collagen derived from the continuous collagenous region of the major triple helical domain. These polypeptides can be prepared by conventional solid phase synthesis and preferably include 15 amino acid residues. As used herein, an all-D polypeptide may include amino acid residues that are not chiral and therefore are in neither the D or the L form (e.g., glycine).

In one embodiment, the formula of the polypeptide is: gly-val-lys-gly-asp-lys-gly-asn-pro-gly-trp-pro-gly-ala-pro. This specific polypeptide formally substantially corresponds to isolated type IV collagen residues1263-1277 from the major triple helical region of the $\alpha$1 chain of type IV collagen, although all the amino acids are in the D-form where appropriate (gly is in neither the L nor the D form). The single letter amino acid code for this polypeptide is GVKGDKGNPGWPGAP. Herein, this specific polypeptide is designated "D-IVH1".

The all-D polypeptide D-IV H1 was assayed for biological activity. It does not efficiently promote the adhesion and spreading of many cell types, and is not a potent attractant for melanoma cell motility. This is in contrast to the all-L form. However, like the all-L form, the all-D form efficiently inhibits tumor cell binding to type IV collagen, tumor cell invasion of basement membranes, and tumor cell metastasis *in vivo*. Also, like the all-L form, the all-D form is highly specific in its cell binding properties. Therefore, it is believed that polypeptides such as D-IVH1 may be useful to (a) inhibit the metastasis and invasion of tumor cells, and (b) target cytotoxic agents to tumor cells. Since it is expected that further hydrolysis of the peptide D-IVH1 *in vitro* or *in vivo* will yield some fragments of substantially equivalent bioactivity, such lower molecular weight peptides are also considered to be within the scope of the present invention.

The present invention also provides peptide-conjugates wherein the all-D form, or the all-L form, of the polypeptides described herein, particularly the IV-H1 peptide (e.g., a peptide incorporating $\alpha$1(IV) residues 1263-1277), is attached (covalently bonded) to a non-peptide moiety, such as a lipophilic $C_{10}$ alkyl "tail" and polyethylene glycol (PEG). Such conjugates inhibit tumor cell binding to type IV collagen.

The polypeptides and peptide-conjugates described herein can also include a cytotoxic agent for selective targeting of tumor cells for therapeutic effect. In such complexes, the cytotoxic agent is covalently bonded to a peptide portion, although it could be covalently bonded to a non-peptide moiety.

The present invention also provides therapeutic methods. For example, the present invention provides a method of inhibiting tumor cell binding (adhesion) to type IV collagen comprising contacting the tumor cell with a polypeptide or peptide-conjugate as described herein. Another method of the present invention involves inhibiting tumor cell invasion of a basement membrane. The method includes modulating the tumor cell with a polypeptide or peptide-conjugate as described herein. The present invention also provides a

method of inhibiting tumor cell metastasis comprising modulating the tumor cell with a polypeptide or peptide-conjugate as described herein. Preferably, each of these methods is carried out *in vivo*. As used herein, "inhibiting" does not necessarily mean complete elimination of the activity, rather it means that the level of the activity (tumor cell binding, invasion, or metastasis) is decreased relative to the level of that activity in the absence of the polypeptide or peptide-conjugate. The term "modulating" means bringing the polypeptide or peptide-conjugate in close proximity to, and preferably so close that it is in contact with, the tumor cell.

**Brief Description of the Drawings**

Figures 1A and 1B show the relative inhibition of M14#5 human melanoma cell adhesion to 10 μg/mL type IV collagen (TIV), fibronectin (FN), laminin (LM), or bovine serum albumin (BSA) by 100 μg/mL of L-IVH1, D-IVH1, or RI-IVH1 (a polypeptide having the sequence pro-ala-gly-pro-trp-gly-pro-asn-gly-lys-asp-gly-lys-val-gly, which is the all-D form synthesized in the reverse order and referred to as "Retro-Inverso"). Cells were preincubated with the peptides for 15 minutes and then added to the wells in the presence of the peptides for a 30-minute incubation period at 37°C. The data represent the means of triplicate points plus or minus the standard errors of the means. Figures 1A and 1B represent different experiments run under the same conditions.

Figure 2A and B show the inhibition of M14#5 human melanoma cell invasion through MATRIGEL by 500 μg/mL (A) or 1 mg/mL (B) of L-IVH1, D-IVH1, or RI-IVH1 (a polypeptide having the sequence pro-ala-gly-pro-trp-gly-pro-asn-gly-lys-asp-gly-lys-val-gly, which is the all-D form synthesized in the reverse order and referred to as "Retro-Inverso"). Cells were mixed with the peptides and then tested for their ability to invade through MATRIGEL basement membrane (obtained from Collaborative Biomedical Products). The data represents the means of triplicate points plus or minus the standard errors of the means.

Figure 3 shows the inhibition of M14#5 human melanoma cell adhesion to 10 μg/mL type IV collagen by D-IVH1(-Y) (closed squares), D-IVH1' (closed circles), D-IVH1(-Y)C10 (open squares), D-IVH1C'10 (open circles), or D-IVH1'PEG (starred circles). Cells were preincubated with the peptides for 15 minutes and then added to the wells in the presence of the peptides for a 60-minute incubation period at 37°C. The data represent the means of triplicate points plus or minus the standard errors of the means.

Figure 4 is a graph showing the inhibition of M14#5 human melanoma cell adhesion to 10 μg/mL type IV collagen by D-IVH1' (closed squares), D-IVH1'C10 (open squares), or D-IVH1'PEG (closed circles). Cells were preincubated with the peptides for 15 minutes and then added to the wells in the presence of the peptides for a 60-minute incubation period at 37°C. The data represent the means of triplicate points plus or minus the standard errors of the means.

**Detailed Description of the Invention**

The structure of the two α1 chains and the single α2 chain of type IV collagen has been the subject of much study. The sequence of the α1 chain is shown in Figure 2 of U.S. Patent No. 5,082,926 (Chelberg et al.). The total number of amino acids per collagen molecule is approximately 4,550, with each 1(IV) chain containing approximately 1,390 amino acids.

The inhibitory activities of IV-H1 synthesized with all-L amino acids (designation L-IVH1), all-D amino acids (designated D-IVH1), and IV-H1 synthesized in reverse sequence order with all-D amino acids (retro-inverso; designated RI-IVH1) were analyzed. The all-D IV-H1 inhibits melanoma cell adhesion to type IV collagen (Figure 1) and invasion of MATRIGEL basement membrane (Figure 2) at least as well as does the all-L form. The retro-inverso form of IV-H1 has only weak inhibitory properties at best. Thus, the present invention provides polypeptides which represent an all-D form of a fragment of the α1 chain of human type IV collagen derived from continuous collagenous region of the major triple helical domain.

Surprisingly, both the all-L and all-D versions of IV-H1 inhibit melanoma cell metastasis *in vivo* (Table 1). Also, the all-D version inhibits spontaneous Lewis lung tumor metastasis. These results are in contrast to that of Nomizu et al., *J. Biol. Chem*, *267*, 14118-14121 (1998), who found that an all-D laminin derived synthetic peptide LAM-L (A chain residues 2097-2108) *increased* murine melanoma cell growth *in vivo* in comparison to no peptide.

The present invention also provides peptide-conjugates, i.e., where a non-peptide moiety is incorporated onto a polypeptide as described above, particularly onto the peptide IV-H1, for the all-D form as well as the all-L form of the polypeptide. Peptide-conjugates are typically created to improve the bioavailability and subsequent half-life of peptide-based drugs *in vivo*. The peptide-conjugates of the present invention have been shown to inhibit adhesion of tumor cells to type-IV collagen, and are believed to provide inhibitory activity with respect to tumor cell invasion of basement membranes and tumor cell metastasis.

Preferably, the non-peptide moieties are typically those that impart some hydrophobic character to the peptide and are not readily hydrolyzed. Preferred non-peptide moieties include alkyl chains (typically, $C_6$-$C_{18}$ alkyls to provide, e.g., monoalkyl tails and dialkyl tails), phospholipids, and polyalkylene glycols. Specific examples include, for example, a lipophilic $C_{10}$ alkyl "tail" and polyethylene glycol (PEG). Such conjugates can be synthesized by methods known in the art, particularly solid phase methods.

In certain specific embodiments, the non-peptide moiety can be any organic group having a long alkyl group (preferably, a linear chain). For example, the organic group can include at least two long alkyl groups (preferably, linear chains) that are capable of forming lipid-like structures. This organic group also includes suitable functional groups for attachment to the peptide portion. Preferably, the organic group is attached to the peptide portion through a linker group having suitable functionality such as ester groups, amide groups, and combinations thereof. Suitable non-peptide moieties can be derived from compounds such as, for example, alkylamines, alkylesters, and

phospholipids.

When lipophilic non-peptide moieties are used, bilayer membrane systems can be formed, where the lipid moiety serves as an anchor for the functional head group to the lipid assembly. For example, such peptide-conjugates may form a great variety of structures in solution including micelles and vesicles. They can also be mixed with vesicle-forming lipids, such as dilauryl phosphatidylcholine, to form stable mixed vesicles with peptide head groups. These can be used as delivery vesicles for the peptide and optionally a cytotoxic agent. For example, a drug targeting system against melanoma cells can be designed using such complexes.

In the examples discussed below, non-peptide moieties were added to one of two forms of all-D IV-H1; one containing just the IV-H1 sequence [designated D-IVH1(-Y)], and one containing the IV-H1 sequence and a *C*-terminal Tyr residue (designated D-IVH1'). $C_{10}$-D-IV-H1 [designated either D-IVH1(-Y)C10 or D-IVH1'C10] and $PEG_{1900}$-D-IV-H1 (designated D-IVH1'PEG) were tested for inhibition of M14 human melanoma cell adhesion to type IV collagen. Both $C_{10}$-D-IV-H1 and $PEG_{1900}$-D-IV-H1 inhibited melanoma cell adhesion to type IV collagen in a dose-dependent fashion (Figure 3). The IV-H1 sequence and the IV-H1 containing a *C*-terminal Tyr residue were tested. There was more effective inhibition when the Tyr was not present (Figure 3).

$C_{10}$-D-IV-H1 [designated D-IVH1'C10] and $PEG_{1900}$-D-IV-H1 (designated D-IVH1'PEG) were subsequently retested for inhibition of M14 human melanoma cell adhesion to type IV collagen. D-IV-H1, $C_{10}$-D-IV-H1, and $PEG_{1900}$-D-IV-H1 all inhibited melanoma cell adhesion to type IV collagen in similar dose-dependent fashions (Figure 4). Thus, adding a conjugate to the D-IV-H1 sequence does not compromise the inhibitory properties of D-IV-H1, and may improve the *in vivo* half-life of this potential therapeutic.

The present invention also provides complexes and methods wherein a cytotoxic agent can be delivered to a cell. That is, the polypeptides or peptide-conjugates described herein can be used to target specific tumor cells, bind thereto, optionally invade the cellular structure, and deliver a cytotoxic

agent. Examples of cytotoxic agents include DNA intercalators, metal chelators, alkylating agents, and membrane disrupting agents. Examples of specific such agents include risin A, dioxorubicin, and mitomycin C.

The complexes (polypeptides and conjugates with or without cytotoxic agents attached thereto) of the present invention can be made by a variety of solid-phase or solution techniques. Although the polypeptides can be prepared by other methods (e.g., solution methods) and then attached to a support material for subsequent coupling with a non-peptide moiety, it is preferred that standard solid-phase organic synthesis techniques, such as solid-phase peptide synthesis (SPPS) techniques be used for preparation of the peptides as well as the conjugates.

Preferably, solid-phase peptide synthesis involves a covalent attachment step (i.e., anchoring) that links the nascent peptide chain to a support material (typically, an insoluble polymeric support) containing appropriate functional groups for attachment. Subsequently, the anchored peptide is extended by a series of addition (deprotection/coupling) cycles that involve adding $N^{\alpha}$-protected and side-chain-protected amino acids stepwise in the C to N direction. Once chain assembly has been accomplished, protecting groups are removed and the peptide is cleaved from the support. Typically, the non-peptide moiety and/or the cytotoxic agent is added to the peptide before the protecting groups are removed.

Typically, SPPS begins by using a handle to attach the initial amino acid residue to a functionalized support material. A handle (i.e., linker) is a bifunctional spacer that, on one end, incorporates features of a smoothly cleavable protecting group, and on the other end, a functional group, often a carboxyl group, that can be activated to allow coupling to the functionalized support material. Known handles include acid-labile p-alkoxybenzyl (PAB) handles, photolabile o-nitrobenzyl ester handles, and handles such as those described by Albericio et al., *J. Org. Chem.*, *55*, 3730-3743 (1990) and references cited therein, and in U.S. Patent Nos. 5,117,009 (Barany) and 5,196,566 (Barany et al.).

For example, if the support material is prepared with amino-functional monomers, typically, the appropriate handles are coupled quantitatively in a single step onto the amino-functionalized supports to provide a general starting point of well-defined structures for peptide chain assembly. The handle protecting group is removed and the C-terminal residue of the $N^{\alpha}$-protected first amino acid is coupled quantitatively to the handle. Once the handle is coupled to the support material and the initial amino acid or peptide is attached to the handle, the general synthesis cycle proceeds. The synthesis cycle generally consists of deprotection of the $N^{\alpha}$-amino group of the amino acid or peptide on the support material, washing, and, if necessary, a neutralization step, followed by reaction with a carboxyl-activated form of the next $N^{\alpha}$-protected amino acid. The cycle is repeated to form the peptide of interest. Solid-phase peptide synthesis methods using functionalized insoluble support materials are well known. See, for example, Merrifield, *J. Am. Chem. Soc.*, *85*, 2149 (1963); Barany and Merrifield, In *Peptides*, Vol. 2, pp. 1-284 (1979); Barany et al., *Int. J. Peptide Protein Res.*, *30*, 705-739 (1987); Fields et al., In *Synthetic Peptides: A User's Guide* (G.A. Grant, Ed.), Chapter 3, pp. 77-183, W.H. Freeman and Co., NY (1992); and Fields et al., *Int. J. Peptide Protein Res.*, *35*, 161-214 (1990).

When SPPS techniques are used to synthesize the polypeptides described herein on the support material, Fmoc methodologies are preferably used. This involves the use of mild orthogonal techniques using the base-labile $N^{\alpha}$-9-fluorenylmethyloxycarbonyl (Fmoc) protecting group. Fmoc amino acids can be prepared using fluorenylmethyl succinimidyl carbonate (Fmoc-OSu), Fmoc chloride, or [4-(9-fluorenylmethyloxycarbonyloxy)phenyl]dimethylsulfonium methyl sulfate (Fmoc-ODSP). The Fmoc group can be removed using piperidine in dimethylformamide (DMF) or N-methylpyrrolidone, or using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in DMF. After Fmoc removal, the liberated $N^{\alpha}$-amine of the supported resin is free and ready for immediate attachment of the non-peptide moiety without an intervening neutralization step. The immobilized conjugate can then be removed, for example, using

trifluoroacetic acid (TFA) at room temperature. Such Fmoc solid-phase peptide synthesis methodologies are well known to one of skill in the art and are discussed in Fields et al., In *Synthetic Peptides: A User's Guide* (G.A. Grant, Ed.), Chapter 3, pp. 77-183, W.H. Freeman and Co., NY (1992); and Fields et al., *Int. J. Peptide Protein Res.*, *35*, 161-214 (1990).

A variety of support materials for preparation of the complexes of the present invention can be used. They can be of inorganic or organic materials and can be in a variety of forms (e.g., membranes, particles, spherical beads, fibers, gels, glasses, etc.). Examples include, porous glass, silica, polystyrene, polyethylene terephthalate, polydimethylacrylamides, cotton, paper, and the like. Examples of suitable support materials are described by Fields et al., *Int. J. Peptide Protein Res.*, *35*, 161-214 (1990); and *Synthetic Peptides: A User's Guide* (G.A. Grant, Ed.), Chapter 3, pp. 77-183, W.H. Freeman and Co., NY (1992). Functionalized polystyrene, such as amino-functionalized polystyrene, aminomethyl polystyrene, aminoacyl polystyrene, p-methylbenzhydrylamine polystyrene, or polyethylene glycol-polystyrene resins can be used for this purpose.

Objects and advantages of this invention are further illustrated by the following examples, but the particular materials and amounts thereof recited in these examples, as well as other conditions and details, should not be construed to unduly limit this invention.

**Synthesis of the Polypeptide**

Methods for the synthesis of peptides have been described extensively previously (C. Fields, et al., *J. Biol. Chem.*, *268*, 14153-14160 (1993); A. Miles et al., *J. Biol. Chem.*, *269*, 30939-30945 (1994); Y.-C. Yu et al., *J. Am. Chem. Soc.*, *118*, 12515-12520 (1996); G. Fields et al., *Synthetic Peptides: A User's Guide*, (Grant, G.A., ed.), pp. 77-183, W. H. Freeman & Co., New York (1992); C. Fields et al., *Biopolymers*, *33*, 1695-1707 (1993); C. Fields et al., *Peptide Res.*, *6*, 39-47 (1993); G. Rao et al., *J. Biol. Chem.*, *269*, 13899-13903 (1994); H. Nagase et al., *J. Biol. Chem.*, *269*, 20952-20957 (1994);

J. Lauer et al., *Lett. Peptide Sci.*, *1*, 197-205 (1995); B. Grab et al., *J. Biol. Chem.*, *271*, 12234-12240 (1996); J. Lauer et al., *J. Med. Chem.*, *40*, 3077-3084 (1997); C. Fields et al., *Anal. Biochem.*, *231*, 57-64 (1995)). These synthetic methods involved solid-phase techniques using Fmoc-amino acids on an ABI 431A peptide synthesizer. For the preparation of peptide-conjugates, either decanoic acid [$CH_3$-$(CH_2)_8$-$CO_2H$, designated $C_{10}$], or PEG of MW 1900 Da was coupled to the resin-bound peptide using *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium hexafluoro-phosphate *N*-oxide (HBTU) as described previously (Y.-C. Yu et al., *J. Am. Chem. Soc.*, *120*, in press).

Peptides and peptide-conjugates were purified using reversed-phase high performance liquid chromatography (RP-HPLC) on a Rainin AutoPrep System. Peptides were purified with a Vydec 218TP152022 $C_{18}$ column (15-20 μm particle size, 300 Angstrom pore size, 250 x 25 mm) at a flow rate of 5.0 ml/minute. The elution gradient was either 0-60% B or 0-100% B in 60 minutes, where A was 0.1% TFA in water and B was 0.1% TFA in acetonitrile. Detection was at 229 nm. Peptide-conjugate purification was achieved using either the method described above or a Vydac 214TP152022 $C_4$ column (15-20 μm particle size, 300 Angstrom pore size, 250 x 22 mm) at a flow rate of 10 ml/minute. The elution gradient was 55-90% B in 20 minutes, where A was 0.05% TFA in water and B was 0.05% TFA in acetonitrile. Detection was at 229 nm. Analytical RP-HPLC was performed on a Hewlett-Packard 1090 Liquid Chromatograph equipped with a Hypersil $C_{18}$ column (5 μm particle size, 120 Angstrom pore size, 200 x 2.1 mm) at a flow rate of 0.3 ml/minute. The elution gradient was 0-60% B in 45 minutes, where A and B were the same as for peptide purification. Diode array detection was at 220, 254, and 280 nm.

Purity and composition of the final compounds was assured by Edman degradation sequence analysis of the peptides and analytical RP-HPLC and laser desorption mass spectrometry (LDMS) of the peptides and peptide-conjugates. Edman degradation sequence analysis was performed on an

Applied Biosystems 477A Protein Sequencer/120A Analyzer. LDMS was performed on a Hewlett Packard matrix-assisted laser desorption time-of-flight mass spectrometer.

To synthesize either a peptide or peptide-conjugate containing a cytotoxic agent, one would need to assemble the toxin, such as the risin A chain, onto the α-amino group of the peptide and the α- or ε-amino group of the peptide-conjugate. For example, the all-D IV-H1 is synthesized, and the risin A chain sequence (Gln-Tyr-Ile-Lys-Ala-Asn-Ser-Lys-Phe-Ile-Gly-Ile-Thr-Glu) is assembled onto the *N*-terminus of the resin-bound IV-H1 sequence by standard solid-phase methods (G. Fields et al., *Synthetic Peptides: A User's Guide* (Grant, G.A. ed.), pp. 77-183, W.H. Freeman & Co., New York (1992)). A spacer such as 6-aminohexaonic acid may or may not be included between the IV-H1 and risin A sequences. Alternatively, for peptide-conjugates, the all-D IV-H1 is synthesized, an Fmoc-Lys(Dde) residue is incorporated (where Dde is 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)-ethyl), the Fmoc group is removed, and the risin A chain sequence is added to the resin-bound peptide. The Dde group is removed with hydrazine (C. Fields et al., *Biopolymers*, *33*, 1695-1707 (1993) and the conjugate (alkyl tail or PEG) is added to the *N*-ε-amino group of the resin-bound peptide. The peptide or peptide-conjugate is then purified and characterized as described above.

**Cell Culture**

Human melanoma cells were cultured in Eagle's minimum essential media supplemented with 10% fetal bovine sera, 1 mM sodium pyruvate, 0.1 mg/mL gentamicin (Boehringer Mannheim, Indianapolis, IN), 50 units/mL penicillin, and 0.05 mg/mL streptomycin. Cells were passaged 8 times and then replaced from frozen stocks of early passage cells to minimize phenotypic drift. All cells were maintained at 37°C in a humidified incubator containing 5% $CO_2$. All media reagents were purchased from Sigma Chemical Co. (St. Louis, MO).

**Labeling of Peptides**

Assays are first performed to quantitate the amount of each peptide adsorbed to the wells after adsorption and rinsing. Synthetic peptides are radiolabeled by reductive methylation using sodium cyanoborohydride and 3H-formaldehyde. By this technique, the ε-amino groups of Lys and the α-amino terminus become labeled. The radiolabeled substrate is added to microtiter wells and incubated overnight. Wells are blocked, then rinsed. Lysis buffer (0.5 M NaOH, 1% SDS) is then used to remove the radioactivity for quantitation.

**Adhesion Assay**

Adhesion of cells was determined as described previously (C. Fields et al., *J. Biol. Chem.*, *268*, 14153-14160 (1993); A. Miles et al., *J. Biol. Chem.*, *269*, 30939-30945 (1994); C. Li et al., *Biochemistry*, *36*, 15404-15410 (1997); J. Lauer et al., *J. Med. Chem.*, *40*, 3077-3084 (1997)). Briefly, peptides were dissolved in 1 mL of water or DMSO-water (1:9), diluted to desired concentrations with PBS, and adsorbed directly onto 96-well polystyrene Immulon 1 plates (Dynatech Laboratories Inc., Chantilly, VA) overnight at 37°C. Nonspecific binding sites were blocked with 2 mg/mL ovalbumin in phosphate buffered saline (PBS) for 2 hours at 37°C. Cells were radiolabeled overnight with 20 μCi/mL Tran $^{35}$S-Label™ (>1000 Ci/mmol specific activity; ICN, Costa Mesa, CA). Cells were released from tissue culture flasks with 37°C PBS containing 0.05% trypsin and 0.53 mM EDTA, then washed several times with PBS. Cells were added to the wells at a density of 50,000 cells/mL in a total volume of 100 μL of the respective cell media containing 2 mg/mL ovalbumin and incubated for 2 hours at 37°C. Wells were washed several times with PBS containing 2 mg/mL ovalbumin and the remaining adherent cells were lysed and radioactivity determined as described (C. Fields et al., *J. Biol. Chem.*, *268*, 14153-14160 (1993); A. Miles et al., *J. Biol. Chem.*, *269*, 30939-30945 (1994); C. Li et al., *Biochemistry*, *36*, 15404-15410 (1997); J. Lauer et al., *J. Med. Chem.*, *40*, 3077-3084 (1997)). Adhesion percentages were based on total counts of radioactivity added to each well.

Competition of cell adhesion assays were performed as described previously (A. Miles et al., *J. Biol. Chem.*, *269*, 30939-30945 (1994); C. Li et al., *Biochemistry*, *36*, 15404-15410 (1997)) using substrate at concentrations which provide ≥50% initial cell adhesion. Cells were preincubated for 30 minutes at 37°C with various concentrations of the inhibitory peptide, then the cells, in the continued presence of the inhibitor, are added to the wells and allowed to adhere for 30 minutes at 37°C.

The invention will be further described by reference to the following detailed example.

**Example 1**

L-IVH1, D-IVH1, and RI-IVH1 were tested for their ability to inhibit metastasis *in vivo* as described previously (I. Saiki et al., *Jpn. J. Cancer Res.*, *84*, 326-335 (1993)). Highly metastatic A375SM human melanoma cells (A375SM melanoma cells supplied by Dr. James B. McCarthy, University of Minnesota, who had originally obtained them from Dr. I.J. Fidler, M.D. Anderson Hospital, Houston, TX) were pre-incubated several different concentrations of L-IVH1, D-IVH1, and RI-IVH1 (Table 1). The cells were then injected into the lateral tail veins of immunocompromised (KSN nude female) mice (Shizuoka Laboratory Animal Center, Hamamatsu, Japan), which had 24 hours prior to this been injected with 20 μL of anti-asialo GM1 antisera (Shizuoda Lavoratory Animal Center, Hamamatsu, Japan). After 50 days, the mice were sacrificed and the number of lung metastatic nodules was quantified in a blinded fashion. The data represent the means of 5 animals/group, plus or minus the standard deviations (SD) of the means. The all-L and all-D versions of IV-H1 were found to inhibit melanoma cell metastasis *in vivo* (Table 1). It was also found that a dose of 100 μg/mouse of D-IVH1, initiated one day after tumor implantation, would inhibit spontaneous Lewis lung tumor metastasis by 50%. These results are in contrast to that of Nomizu et al., *J. Biol. Chem*, *267*, 14118-14121 (1998), who found that an all-D laminin derived synthetic peptide LAM-L (A chain residues 2097-2108) *increased* murine melanoma cell growth *in vivo*.

**Table 1: Effects of IV-H1 peptide variants on experimental lung metastasis produced by intravenous injection of human A375SM melanoma cells.**

| Peptide | Dose (μg/mouse) | Lung metastases on day 50 mean + SD (range) |
|---|---|---|
| Control (PBS) | 0 | 90 + 15 (80-117) |
| L-IVH1 | 10 | 93 + 11 (81-107) |
| L-IVH1 | 100 | 50 + 12 (36-62) |
| L-IVH1 | 1000 | 16 + 13 (4-34) |
| D-IVH1 | 10 | 88 + 12 (65-96) |
| D-IVH1 | 100 | 43 + 10 (31-54) |
| D-IVH1 | 1000 | 31 + 10 (21-46) |
| RI-IVH1 | 10 | 86 + 12 (72-102) |
| RI-IVH1 | 100 | 84 + 8 (76-96) |
| IR-IVH1 | 1000 | 64 + 9 (54-77) |

The inhibitory behaviors of D-IVH1 have also been examined by synthesizing several peptide-conjugates, i.e., where a non-peptide moiety is incorporated onto IV-H1. Peptide-conjugates are created to improve the bioavailability and subsequent half-life of peptide-based drugs *in vivo*. Two conjugates have been studied: a lipophilic $C_{10}$ alkyl "tail" and polyethylene glycol (PEG). Conjugates were added to one of two forms of all-D IV-H1; one containing just the IV-H1 sequence [designated D-IVH1(-Y)], and one containing the IV-H1 sequence and a *C*-terminal Tyr residue (designated D-IVH1'). The $C_{10}$ alkyl tail was coupled to resin-bound all-D IV-H1 and the product purified and characterized using methods described previously (P. Berndt et al., *J. Am. Chem. Soc.*, *117*, 95159-9522 (1995); and Y.C. Yu, *J. Am. Chem. Soc.*, *118*, 12515-12520 (1996)). PEG of MW 1900 was coupled to resin-bound all-D IV-H1 and the product purified and characterized as described

previously (P. Berndt et al., *J. Am. Chem. Soc.*, *117*, 9515-9522 (1995); Y.C. Yu et al., *J. Am. Chem. Soc.*, *118*, 12515-12520 (1996); Y.A. Lu et al., *Peptide Res.*, *6*, 140-146 (1993); and Y.C. Yu et al., *J. Am. Chem. Soc.*, *120*, 9979-9987 (1998)). $C_{10}$-D-IV-H1 [designated either D-IVH1(-Y)C10 or D-IVH1'C10] and $PEG_{1900}$-D-IV-H1 (designated D-IVH1'PEG) were tested for inhibition of M14 human melanoma cell adhesion to type IV collagen using an assay previously described (A.J. Miles et al., *J. Biol. Chem.*, *269*, 30939-30945 (1994)). Both $C_{10}$-D-IV-H1 and $PEG_{1900}$-D-IV-H1 inhibited melanoma cell adhesion to type IV collagen in a dose-dependent fashion (Figure 3). The IV-H1 sequence and the IV-H1 containing a *C*-terminal Tyr residue were tested. There was more effective inhibition when the Tyr was not present (Figure 3).

$C_{10}$-D-IV-H1 [designated D-IVH1'C10] and $PEG_{1900}$-D-IV-H1 (designated D-IVH1'PEG) were subsequently retested for inhibition of M14 human melanoma cell adhesion to type IV collagen. D-IV-H1, $C_{10}$-D-IV-H1, and $PEG_{1900}$-D-IV-H1 all inhibited melanoma cell adhesion to type IV collagen in similar dose-dependent fashions (Figure 4). Thus, adding a conjugate to the D-IV-H1 sequence does not compromise the inhibitory properties of D-IV-H1, and may improve the *in vivo* half-life of this potential therapeutic.

The complete disclosures of the patents, patent documents, and publications cited herein are incorporated by reference in their entirety as if each were individually incorporated. Various modifications and alterations to this invention will become apparent to those skilled in the art without departing from the scope and spirit of this invention. It should be understood that this invention is not intended to be unduly limited by the illustrative embodiments and examples set forth herein and that such examples and embodiments are presented by way of example only with the scope of the invention intended to be limited only by the claims set forth herein as follows.

## WHAT IS CLAIMED IS:

1. A polypeptide having an amino acid sequence which is a fragment of the continuous collagenous region of the major triple helical domain of the α1 chain of type IV collagen, wherein the polypeptide is in the all D-form.

2. The polypeptide of claim 1 wherein the amino acid sequence corresponds substantially to amino acid residues 1263 through 1277 of the continuous collagenous region of the major triple helical domain of the α1 chain of type IV collagen.

3. The polypeptide of claim 2 having 15 amino acid residues in the D-form where appropriate.

4. The polypeptide of claim 3 having the sequence gly-val-lys-gly-asp-lys-gly-asn-pro-gly-trp-pro-gly-ala-pro.

5. The polypeptide of claim 1 further comprising a cytotoxic agent covalently bonded thereto.

6. The polypeptide of claim 1 which inhibits binding of tumor cells to type IV collagen.

7. The polypeptide of claim 1 which inhibits tumor cell invasion into basement membranes.

8. The polypeptide of claim 1 which inhibits tumor cell metastasis.

9. A peptide-conjugate comprising a polypeptide fragment of the continuous collagenous region of the major triple helical domain of the

α1 chain of type IV collagen covalently bonded to a non-peptide moiety.

10. The peptide-conjugate of claim 9 wherein the polypeptide fragment is in the all D-form.

11. The peptide-conjugate of claim 9 wherein the polypeptide fragment is in the all L-form.

12. The peptide-conjugate of claim 9 wherein the amino acid sequence of the polypeptide fragment corresponds substantially to amino acid residues 1263 through 1277 of the continuous collagenous region of the major triple helical domain of the α1 chain of type IV collagen.

13. The peptide-conjugate of claim 12 having 15 amino acid residues in the D-form where appropriate.

14. The peptide-conjugate of claim 13 having the sequence gly-val-lys-gly-asp-lys-gly-asn-pro-gly-trp-pro-gly-ala-pro.

15. The peptide-conjugate of claim 9 further comprising a cytotoxic agent covalently bonded thereto.

16. A method of inhibiting tumor cell binding to type IV collagen comprising contacting the tumor cell with a polypeptide of claim 1 or a peptide-conjugate of claim 9.

17. A method of inhibiting tumor cell invasion of a basement membrane comprising modulating the tumor cell with a polypeptide of claim 1 or a peptide-conjugate of claim 9.

18. A method of inhibiting tumor cell metastasis comprising modulating the

tumor cell with a polypeptide of claim 1 or a peptide-conjugate of claim 9.

19. The method of any of claims 16-18 which is carried out *in vivo*.

Figure 1A

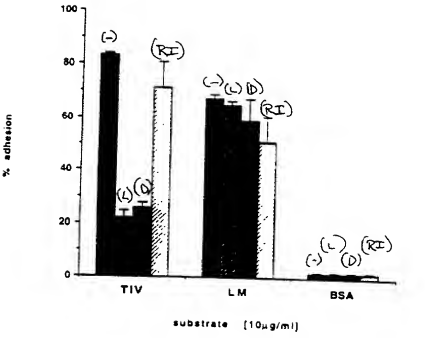



Figure 1B

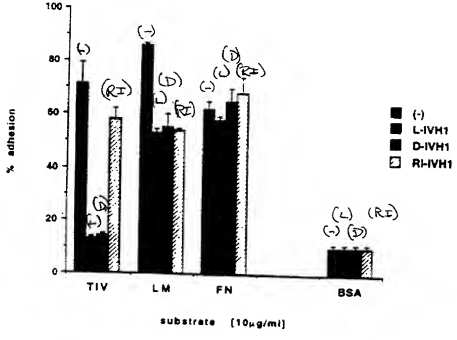


Figure 2A

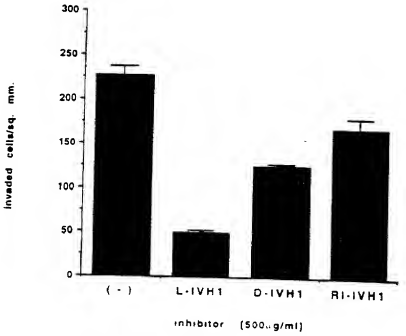
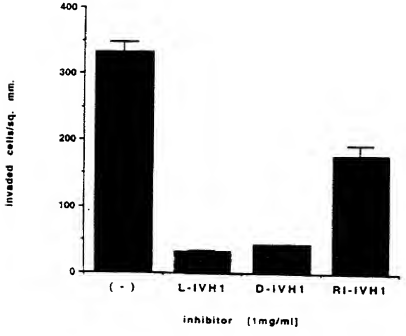
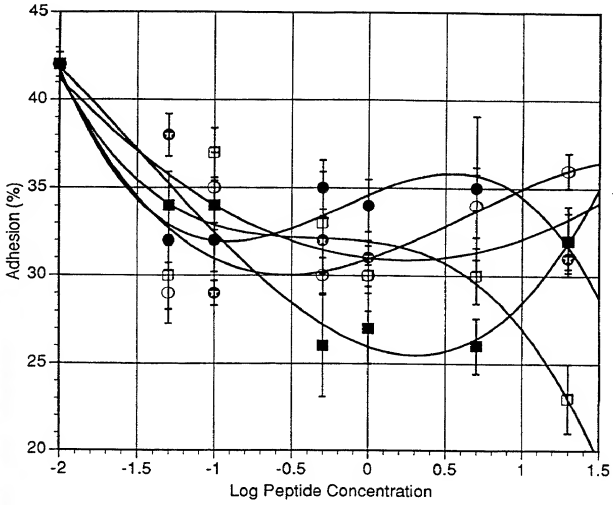
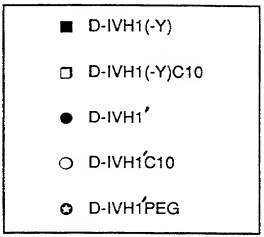



Figure 2B





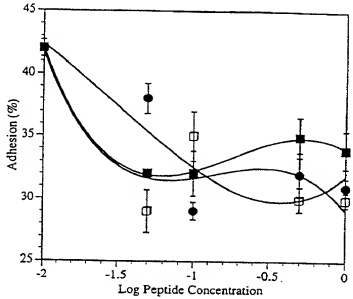



Figure 4

Docket No: 110.0068 0101

## DECLARATION AND POWER OF ATTORNEY

We, Gregg B. Fields and James B. McCarthy, declare that: (1) our respective citizenships and residence/post office addresses are indicated below; (2) we have reviewed and understand the contents of the specification identified below, including the claims, as amended by any amendment specifically referred to herein, (3) we believe that we are the original, first, and joint inventors of the subject matter in

**INHIBITION OF TUMOR CELL ADHESION TO TYPE IV COLLAGEN**

Filing Date: 18 April 2000 Serial No.: 09/529,691

described and claimed therein and for which a patent is sought; and (4) we hereby acknowledge our duty to disclose to the United States Patent and Trademark Office all information known to us to be material to the patentability as defined in Title 37, Code of Federal Regulations, §1.56.*

We hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate listed below, or §365(a) of any PCT international application which designates at least one country other than the United States of America listed below, and have also identified below any foreign application for patent or inventor's certificate, or any PCT international application having a filing date before that of the application on the basis of which priority is claimed:

a. X no such applications have been filed.
b. __ such applications have been filed as follows:

| FOREIGN APPLICATION(S), IF ANY, CLAIMING PRIORITY UNDER 35 USC §119(a)-(d), §365(a), and/or §365(b) | | | |
|---|---|---|---|
| **COUNTRY** | **APPLICATION NUMBER** | **DATE OF FILING (day, month, year)** | **DATE OF ISSUE (day, month, year)** |
| | | | |
| | | | |

| ALL FOREIGN APPLICATIONS, IF ANY, FILED BEFORE THE PRIORITY APPLICATION(S) | | | |
|---|---|---|---|
| **COUNTRY** | **APPLICATION NUMBER** | **DATE OF FILING (day, month, year)** | **DATE OF ISSUE (day, month, year)** |
| | | | |
| | | | |

* Title 37, Code of Federal Regulations, §1.56 is reproduced on the attached page.

§ 1.56 Duty to disclose information material to patentability.

(a) A patent by its very nature is affected with a public interest. The public interest is best served, and the most effective patent examination occurs when, at the time an application is being examined, the Office is aware of and evaluates the teachings of all information material to patentability. Each individual associated with the filing and prosecution of a patent application has a duty of candor and good faith in dealing with the Office, which includes a duty to disclose to the Office all information known to that individual to be material to patentability as defined in this section. The duty to disclose information exists with respect to each pending claim until the claim is cancelled or withdrawn from consideration, or the application becomes abandoned. Information material to the patentability of a claim that is cancelled or withdrawn from consideration need not be submitted if the information is not material to the patentability of any claim remaining under consideration in the application. There is no duty to submit information which is not material to the patentability of any existing claim. The duty to disclose all information known to be material to patentability is deemed to be satisfied if all information known to be material to patentability of any claim issued in a patent was cited by the Office or submitted to the Office in the manner prescribed by §§1.97(b)-(d) and 1.98. However, no patent will be granted on an application in connection with which fraud on the Office was practiced or attempted or the duty of disclosure was violated through bad faith or intentional misconduct. The Office encourages applicants to carefully examine:

(1) Prior art cited in search reports of a foreign patent office in a counterpart application, and
(2) The closest information over which individuals associated with the filing or prosecution of a patent application believe any pending claim patentably defines, to make sure that any material information contained therein is disclosed to the Office.

(b) Under this section, information is material to patentability when it is not cumulative to information already of record or being made of record in the application, and

(1) It establishes, by itself or in combination with other information, a prima facie case of unpatentability of a claim; or
(2) It refutes, or is inconsistent with, a position the applicant takes in:
  (i) Opposing an argument of unpatentability relied on by the Office, or
  (ii) Asserting an argument of patentability.

A prima facie case of unpatentability is established when the information compels a conclusion that a claim is unpatentable under the preponderance of evidence, burden-of-proof standard, giving each term in the claim its broadest reasonable construction consistent with the specification, and before any consideration is given to evidence which may be submitted in an attempt to establish a contrary conclusion of patentability.

(c) Individuals associated with the filing or prosecution of a patent application within the meaning of this section are:

(1) Each inventor named in the application;
(2) Each attorney or agent who prepares or prosecutes the application; and
(3) Every other person who is substantively involved in the preparation or prosecution of the application and who is associated with the inventor, with the assignee or with anyone to whom there is an obligation to assign the application.

(d) Individuals other than the attorney, agent or inventor may comply with this section by disclosing information to the attorney, agent, or inventor.

Docket No: 110.0068.0101

## DECLARATION AND POWER OF ATTORNEY

We, Gregg B. Fields and James B. McCarthy, declare that: (1) our respective citizenships and residence/post office addresses are indicated below; (2) we have reviewed and understand the contents of the specification identified below, including the claims, as amended by any amendment specifically referred to herein, (3) we believe that we are the original, first, and joint inventors of the subject matter in

**INHIBITION OF TUMOR CELL ADHESION TO TYPE IV COLLAGEN**

Filing Date: 18 April 2000 Serial No.: 09/529,691

described and claimed therein and for which a patent is sought; and (4) we hereby acknowledge our duty to disclose to the United States Patent and Trademark Office all information known to us to be material to the patentability as defined in Title 37, Code of Federal Regulations, §1.56.*

We hereby claim foreign priority benefits under Title 35, United States Code, §119(a)-(d) or §365(b) of any foreign application(s) for patent or inventor's certificate listed below, or §365(a) of any PCT international application which designates at least one country other than the United States of America listed below, and have also identified below any foreign application for patent or inventor's certificate, or any PCT international application having a filing date before that of the application on the basis of which priority is claimed:

a. X no such applications have been filed.
b. __ such applications have been filed as follows:

| FOREIGN APPLICATION(S), IF ANY, CLAIMING PRIORITY UNDER 35 USC §119(a)-(d), §365(a), and/or §365(b) | | | |
|---|---|---|---|
| **COUNTRY** | **APPLICATION NUMBER** | **DATE OF FILING (day, month, year)** | **DATE OF ISSUE (day, month, year)** |
| | | | |
| | | | |

| ALL FOREIGN APPLICATIONS, IF ANY, FILED BEFORE THE PRIORITY APPLICATION(S) | | | |
|---|---|---|---|
| **COUNTRY** | **APPLICATION NUMBER** | **DATE OF FILING (day, month, year)** | **DATE OF ISSUE (day, month, year)** |
| | | | |
| | | | |

* Title 37, Code of Federal Regulations, §1.56 is reproduced on the attached page

We hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below.

a. ___ no such applications have been filed.
b. X such applications have been filed as follows:

| PROVISIONAL APPLICATION(S), IF ANY, UNDER 35 USC §119(e) | |
|---|---|
| **APPLICATION NUMBER** | **DATE OF FILING (day, month, year)** |
| 60/062,617 | 22 October 1997 |
| 60/062,716 | 22 October 1997 |

We hereby claim the benefit under Title 35, United States Code, §120 of any United States applications or §365(c) of any PCT international application(s) designating the United States of America, listed below. Insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT international application in the manner provided by the first paragraph of Title 35, United States Code, §112, we acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56 which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

a. ___ no such applications have been filed.
b. X such applications have been filed as follows:

| APPLICATION NUMBER | DATE OF FILING (day, month, year) | STATUS (patented, pending, abandoned) |
|---|---|---|
| PCT/US98/22405 | 22 October 1998 | Pending |
| | | |

We hereby appoint Ann M. Mueting (Reg. No. ~~33,977~~), Kevin W. Raasch (Reg. No. ~~35,651~~), Mark J. Gebhardt (Reg. No. ~~35,518~~), Victoria A. Sandberg (Reg. No. ~~41,287~~), David L. Provence (Reg. No. ~~43,022~~), Matthew W. Adams (Reg. No. ~~43,459~~), and Loren D. Albin (Reg. No. ~~37,763~~) our attorneys and agents with full powers (including the powers of appointment, substitution, and revocation) to prosecute this application and any division, continuation, continuation-in-part, reexamination, or reissue thereof, and to transact all business in the United States Patent and Trademark Office connected therewith.

Please direct all correspondence in this case to:

Attention: Ann M. Mueting
Mueting, Raasch & Gebhardt, P.A.
P.O. Box 581415
Minneapolis, MN 55458-1415
Telephone No. (612) 305-1220
Facsimile No. (612) 305-1228

The undersigned declare further that all statements made herein of their own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Wherefore, we pray that Letters Patent be granted to us for the invention described and claimed in the specification identified above and we hereby subscribe our names to the foregoing specification and claims, Declaration and Power of Attorney, on the date indicated below.

7/18/00

Name: Gregg B. Fields Date
Citizenship: United States of America
Residence/Post Office Address:
22709 Pickerel Circle
Boca Raton, Florida 33428 FL
United States of America

Name: James B. McCarthy Date
Citizenship: United States of America
Residence/Post Office Address:
2555 - 37th Avenue South
Minneapolis, Minnesota 55406
United States of America

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Name: Gregg B. Fields Date
Citizenship: United States of America
Residence/Post Office Address:
22709 Pickerel Circle
Boca Raton, Florida 33428
United States of America

8/3/00

Name: James B. McCarthy Date
Citizenship: United States of America
Residence/Post Office Address:
2555 - 37th Avenue South
Minneapolis, Minnesota 55406
United States of America

§ 1.56 Duty to disclose information material to patentability.

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